WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Structural and Functional Assessments for Evaluating Elevated Nutrient Levels in Maryland Streams

Focus Categories: NU, WQL, and ECL

Keywords: Bioindicators, Benthos, Functional Measurements, Leaf Processing, Nitrogen, Phosphorus, Water Quality

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Congressional District: 5^{th}

Statement of Critical Regional or State Water Problems:

There is an ongoing effort by both federal and state agencies to better understand the effects of elevated nutrients and their associated environmental impacts. Currently, the US Environmental Protection Agency in conjunction with State agencies are working to establish nutrient criteria for rivers and streams throughout the United States. It is crucial to have effective tools to accurately assess the environmental impacts of these anthropogenic nutrient loads, and to predict environmental trends produced by the continued growth in our population. Agriculture in Maryland's Coastal Plain results in elevated levels of phosphorus and nitrogen in running waters that eventually impact the Chesapeake Bay. Best management practices have been implemented in agricultural productions throughout Maryland to abate some of the problems associated with non-point source pollution and its effect on the State's surface and ground water supplies. In particular, no-till agriculture has reduced much of the erosion problem and helped to reduce the amount of irrigation necessary in the crop fields. Timed fertilizer applications and better understanding of nutrient utilization by crops has reduced the excessive fertilizer loadings on fields. Although these practices have assisted in reducing some of the contaminant loads to the stream, certain contaminants continue to appear in both the surface water and groundwater. Of particular interest is the phosphorus flux into the stream from the watershed.

Over the past year, stream samples collected by us from the Nassawango Creek on the Eastern Shore of Maryland have illustrated elevated phosphorus levels and occasionally increased nitrate-nitrogen levels. Baseline data collected on both this watershed and a more pristine stream in southern Maryland, the Nanjemoy Creek, indicate very different nutrient loads and differing benthic communities. A better understanding of the effects of phosphorus loading in streams can be gained be studying these two stream systems in concert. This project will assess how functional measurements, like organic material processing, is effected by elevated nutrient levels and compare these findings to established structural biotic community tools already in practice. This information can provide a better understanding of the Coastal Plain areas of Maryland where heavy agricultural practices and chicken rearing productions contribute to the potential non-point source loadings of the watershed.

Statement of Results or Benefits:

The expected results of this project will be a better understanding of the cause-effect relationships between elevated nutrient levels and the organic material breakdown capability of a stream system. It will study the interactions between the biotic community structure and its capacity to function under different aquatic chemical conditions. Elevated nutrients, with specific emphasis on phosphorus, will be the focal point of the studies. The project will develop a preliminary stream assessment tool based on functional responses with an analysis of its effectiveness in two watersheds. In addition, comparison of this new approach to established tools already in use will be completed. The work done in this study can be used to construct a better assessment tool for biological monitoring and classifying the impacts on stream systems. Knowledge of how the biotic community processes organic materials under elevated nutrient conditions will provide another dimension of understanding, and will increase the variety and number of tools available for State and local agencies making resource management decisions.

Another potential benefit is that this study may provide additional information on the coastal plain region of Maryland. This new data will contribute to both the Maryland Department of Natural Resources database for these streams, as well as the United States Environmental Protection Agencies (US EPA) National Nutrient Strategy. The US EPA is in the process of establishing region specific nutrient criteria for rivers and streams (US EPA 1998). Augmenting the available databases will improve the understanding about the specific stream conditions for each of the different regions.

Nature, Scope, and Objectives:

Agriculture in Maryland’s Coastal Plain results in elevated levels of phosphorus and nitrogen in running waters that eventually impact the Chesapeake Bay. Procedures for measuring the impact of nutrients on stream ecosystems have emphasized structural assessment tools, such as species diversity, taxa richness, or the Ephemeroptera, Plecoptera, Trichoptera (EPT) index. Here, we propose one-year study to determine the utility of functional assessment tools (e.g., metrics representing leaf decomposition rates) as measures of ecosystem integrity.

The objectives of this project are:

1. To measure leaf decomposition in coastal plain streams in agricultural and non-agricultural regions in Maryland;

2. To compare leaf decomposition rates to structural indices of water quality; and

3. To measure the functional response of stream biota to the addition of phosphorus and nitrogen.

There are several structural tools already in use that classify a water system based on chemical analyses, the abundance and diversity of biological organisms, and physical characteristics of the river or stream. In the past, water quality tests depended primarily on chemical assessments to characterize lotic systems. There are two distinct advantages to this method: 1) chemical analyses reflect point source contamination problems, and 2) they provide highly quantitative regulatory levels for agencies to monitor. However, in recent years there has been a growing trend to use biological monitoring techniques due to their greater sensitivity to a wide range of pollutants and their early warning capabilities.

Rosenberg and Resh (1993) provide a detailed description and evaluation of the utility of biological monitoring methods, with particular attention given to methods based on sampling benthic macroinvertebrates. Unlike chemical data, which provides water quality information at a discrete point in time, biological organisms are long-term integrators of environmental stresses. Different species of organisms exhibit a range of responses to environmental impacts. Macroinvertebrates in particular provide a very good indicator tool of environmental degradation. They are widespread and are relatively sessile in their aquatic phase, and can be inexpensively sampled. These organisms can be used for laboratory studies, including toxicological, physiological, behavioral, or functional assessments, in order to better understand a particular contaminant's effect (Rosenberg and Resh 1993).

The Maryland Department of Natural Resources has created a region specific index of biological integrity to assess water quality and potential impacts across the State of Maryland (Maryland Biological Stream Survey 1997). The strength of this technique is its combination of multiple metrics in order to develop a greater picture of the stream or river condition (Karr and Chu 1999). The MBSS has also included a benthic macroinvertebrate metric based on the Hilsenhoff's biotic index to assist in rapid biological assessments in aquatic systems (Hilsenhoff 1988). Both of these measurement tools provide a static view of the aquatic conditions focusing on the structural components of the system. The techniques utilize the presence or absence of specific organisms as well as their relative abundance to identify and classify the degradation in an aquatic environment.

Other measurable characteristics of streams that can increase our understanding of environmental impacts may be found in the functions or streams, such as nutrient processing. Such functions may be expected to change in predictable ways when the stream is exposed to contaminants. Understanding how the biotic organisms process organic material, for example, can expand our ability to assess impairments by measuring how well the organisms are breaking down the materials in a given stream system. The dynamic view of assessing the system function under impacted conditions could conceivably become the most critical tool in managing the resource. At a minimum, the functional component will provide a greater understanding of how to assess environmental degradation of a stream or river. This approach provides information on the production of the aquatic system (phytoplankton and zooplankton), the processing of organic materials (the uptake of both allochthonous and autochthonous materials), and the transportation of materials. For these reasons, we intend to develop this functional approach to be used in concert with the structural methods currently used. The development of such assessment tools will provide the resource manager with more comprehensive information regarding detrimental nutrient levels, and will enhance the scientific information available for environmental policy-makers.

Methods:

We propose a one year effort to develop a better assessment tool for understanding of the fate and transport of agricultural nutrients within the surface water. The study intends to link the effects of elevated nutrients to changes in functional aspects of the stream system. It involves the following efforts:

A. <u>To measure leaf decomposition in streams representing agricultural and non-agricultural regions in Maryland</u>

Here, our objective is to test for variations in leaf pack processing between sites within the same watershed, as well as between watersheds. We plan to perform both baseline chemical and physical tests as well as field leaf decomposition studies for this investigation. Monitoring of selected streams on the agricultural impacted Eastern Shore and in the more pristine forested streams of southern Maryland will help to identify chemical, physical, and structural differences between the two environments. We have collected water chemistry, benthic macroinvertebrate samples, and hydrologic data over the past year on two different watersheds in Maryland: the Nassawango Creek south of Salisbury and the Nanjemoy Creek in Southern Maryland. Table 1 illustrates the suite of physical and chemical parameters regularly monitored at our field sites. These tests are performed on a monthly basis at 5 sample sites within each stream system.

The study sites on the Eastern Shore of Maryland have yielded elevated phosphorus levels, on the order of 170-2750 ug PO_4-P/L. These extremely high ambient conditions warrant studies focused on the biotic community and their functional capacity under an environmentally degraded scenario. The study sites in Southern Maryland have not shown such high phosphorus levels, 20-940 ug PO_4-P/L, and therefore can be used as a comparison. This baseline data will provide the foundation for our further investigation into the effects of phosphorus on the stream system and the development of potential monitoring tools.

Leaf decomposition studies will be performed in the field to examine relationships between the stream phosphorus levels and the biotic processing of the leaf matter. These studies will be performed six times during the year. We plan to use tube traps containing 2.5 grams of desiccated red maple leaves. The tube traps will be constructed of PVC tubing with a coarse holes on the upstream side to allow macroinvertebrate entry and fine mesh on the downstream end to prevent leaf matter loss. The tube traps will be secured to bricks and placed on the stream bottom. There will be 8 replicates at each site. The traps will be collected after 21 days to process. The leaves will then be rinsed. After sample dry weights will be measured, followed by the samples being ashed using a muffle furnace. We will calculate the proportion of organic matter decomposed using these weights and compare these values to pre-determined organic matter proportions for maple leaves. This comparison will provide the percentage organic matter loss due to decomposition within the stream environment. These field trials coupled with the monitoring data may illustrate other potential factors influencing the stream function.

B. <u>To compare leaf decomposition rates to structural indices of water quality</u>

The objective is to compare structural and functional indices in order to assess the effectiveness of a new environmental stress indicator. The first step is to gather structural information on the two watersheds. We use artificial leaf packs to collect the macroinvertebrates six time within the year. Five grams of desiccated red maple leaves are bound to a brick and left in the stream for 30 days. We use 8 replicate leaf samples at each site. These leaves are then collected from the field and preserved at 4^o Celsius until processed. The leaves are rinsed in a pan and the macroinvertebrates are trapped by filtering the water through a 425 micrometer mesh size sieve. Each sample is then labeled and preserved in Kahle's solution. The macroinvertebrates are then identified to genus level. Number of taxa, number of individuals, and various other community indices [e.g., the Ephemeroptera, Plecoptera, Trichoptera (EPT) index] will be calculated. This data will assist in defining the community structure present as well as provide information as to which organism could best be used in laboratory bioassays.

Regression analysis will then be performed to assess the differences between the structural and functional measurements. The monthly sampling will continue to provide a longitudinal database that will then be analyzed to compare the decomposition results with the expected structural classification of the stream. This comparison will be based on established models, such as the Maryland Biological Stream Survey (MBSS). The State of Maryland has established regional specific metrics to assess the stream conditions in the different ecoregions within the State (Maryland Department of Natural Resources 1998). The MBSS will provide a good comparison tool to use with these decomposition trials (Maryland Department of Natural Resources 1997). The two different methods of classification will then be compared using multivariate statistics.

C. <u>To measure the functional response of stream biota to the addition of phosphorus and nitrogen</u>

The objective is to identify the effects of varied nutrient concentrations, both phosphorus and nitrogen, on the different biological trophic levels, as well as the effects on the decomposition of the allochthonous leaf matter. This will be accomplished by constructing artificial stream environments and subjecting known organisms to different nutrient enrichment levels. The artificial stream environment consists of 200 ml of natural stream water, collected immediately before each test, in a 250 ml Erlenmeyer flask equipped with a glass tube to supply air under pressure. The air maintains a water current and high oxygen levels comparable to a riffle area of a natural stream. Effects of elevated nutrients will be tested using bacteria and macroinvertebrates. Both of these organisms use the leaf material as a food source. We plan to use *Pycnopsyche sp.*(Trichoptera: Limnephilidae) for the macroinvertebrate toxicology tests to assess nutrient effects on primary production. Pycnopsyche sp. was selected as an appropriate test species due to its presence in the Nanjemoy Creek as well as its functional feeding. It is a shredding macroinvertebrate which breaks down the course particulate organic matter (CPOM) to fine particulate organic matter (FPOM). This processing of organic matter leads to further decomposition by microbial consumers (Hauer and Lamberti 1996).

Three experiments will be conducted with *Pycnopsyche sp.* and bacteria present and then it will be repeated with only the bacteria present. For each, at least four treatments with six replicates will be established with a control, a high, a medium, and a low concentration of nutrients. The water will be spiked with either phosphorus or nitrogen and then with both. Before and after each test red maple leaf discs will be weighed and leaf area determined by a CI-400 CIAS system (CID, Inc.). Leaf discs will also be ashed to determine leaf decomposition rates. Bacterial respiration will be measured using a respirometer to assess their activity level and any potential toxicological effects. Laboratory trials will assist in honing in on individual parameters and their corresponding toxicological effects. Understanding how these organisms are affected by different nutrient concentrations will provide insight into the functional capacity of the stream system. These experiments will be analyzed using ANOVA and regression analysis. Lastly, continued monthly monitoring of the stream water chemistry and hydrologic parameters coupled with laboratory bioassays will help to delineate how elevated nutrients effect the stream system. Multivariate analyses (e.g., principle components, factor analysis) will be conducted to relate decomposition rates to physical, and chemical conditions.

Table 1. Stream Parameters Analysis	
Analysis	*Method*
Total Phosphorus	Hach DR/890 Colorimeter
Reacted Phosphorus	Hach DR/890 Colorimeter
Nitrate/Nitrite	Hach DR/890 Colorimeter
Fluoride	Hach DR/890 Colorimeter
Alkalinity	Hach Universal Digital Titrator
Hardness	Hach Universal Digital Titrator
Turbidity	LaMotte Turbidity in water test kit
Water temperature	Corning Check Mate 90 sensor
Dissolved Oxygen	Corning Check Mate 90 sensor
PH	Corning Check Mate 90 sensor
Conductivity	Corning Check Mate 90 sensor
Light Availability	Decagon Sunfleck Ceptometer
Stream flow / Discharge	Marsh-McBirney flow meter

Project schedule:

Table 2 illustrates the milestone schedule. Field data will be collected throughout the duration of the project. The final report will be complete March 2001.

Project Elements	A00	M00	J00	J00	A00	S00	O00	N00	D00	J00	F00	M00
1. Field data collection and analysis	—	—	—	—	—	—	—	—	—			
2. Indices comparison					—	—	—	—	—			
3. Laboratory bioassays and analysis		—	—	—	—	—	—	—	—			

Facilities:

Presently, two watersheds have been selected as field study sites. Each of these sites is located on Nature Conservancy lands with readily available access. There is a US Geological Survey stream gauging station located at one of the Nassawango Creek sample sites. This enables us to extend our hydrologic database beyond our sample dates in order to better assess the hydrologic impacts on the nutrient loading.

The University of Maryland Department of Entomology has excellent research facilities for conducting the proposed research. Dr. Lamp's laboratory facilities are equipped for field experiments as well as laboratory stream simulations with benthic macroinvertebrates, algae, and bacteria. There are dissection microscopes, stream biotic samplers, rearing cages, microbalances, muffle furnace, and Percival growth chambers which will assist in the aquatic insect studies. Also, the lab is equipped with analytical instrumentation for studying water chemistry parameters. Field tools to assess both the water chemistry and hydrologic parameters, such as discharge are also available. The Department has both shared and personal computer facilities for data management and statistical analysis and internet access to the USGS gauging station database.

Related Research

There have been multiple studies performed in the past focusing on leaf decomposition and the detrital food web (Cummins et al. 1973, Elwood et al. 1981, Howarth et al. 1976, Mullholland et al. 1985, Newbold et al. 1983, Petersen and Cummins 1974, Suberkropp et al. 1976, and Triska and Sedell 1976). Several of these studies illustrate the relationship of how different biotic trophic levels play a crucial role in the decomposition process. Particular attention is focused on the bacteria and their role in the utilization of the organic matter within the stream (Fuss and Smock 1996, Kaplan and Bott 1983, Koetsier et al. 1997, and Suberkropp and Klug 1976). The macroinvertebrate's role in the breakdown of allochthonous materials is illustrated in several experiments (Mulholland et al. 1985, Mulholland et al. 1983, and Cummins et al. 1979). Many of these efforts have tried to explain a part of the stream's ability to cycle nutrients through the decomposition and utilization of both leaf matter and the uptake by primary producers, bacteria and benthic macroinvertebrates.

One of the studies in particular assessed primary production, bacterial activity, and leaf decomposition by macroinvertebrates (Elwood et al. 1981). They observed that streams with artificially elevated phosphorus levels (up to 450 ug PO4-P/L) had increased nitrogen and phosphorus levels in leaf packs, increased respiration rates of leaf discs, and increased mass loss of leaves. In addition, the algal biomass was augmented by the increased phosphorus inputs. These findings suggest that the increase in phosphorus influences both the primary and secondary trophic levels in the aquatic food web (Elwood et al. 1981). However, another study illustrated that it was a combination of nitrogen and phosphorus that caused some of these changes in processing (Howarth et al. 1976).

This project can help to elucidate these discrepancies by testing both the structural and functional approaches. In addition, previous studies looked at artificial stream systems and nutrient spiked streams to assess elevated phosphorus effects. Our project will be able to provide additional information in three areas. First, the Eastern Shore study sites have elevated phosphorus at approximately one and one half orders of magnitude higher than those spiked in Elwood's 1981 study. These sites will help to field test some of the conclusions found in previous works. Second, this information will be specific to the coastal plains environment of Maryland as opposed to the upland area studies previously mentioned. Last, evaluating nutrient effects and limitations specific to a region will serve to better understand the functional processes of coastal plain streams.

This project will compare the functional measurements with the current indices in use. The Maryland Biological Stream Survey (MBSS) provides a region-specific database, which can be used as one of the structural comparisons. The MBSS was conducted State-wide and a number of reference sites selected under this program will give a good points of contrast with our agriculturally impacted sites (Maryland Biological Stream Survey 1997). The survey incorporates Hilsenhoff's rapid biological assessment. Hilsenhoff (1988) bases his assessment tool on families of benthic macroinvertebrates and their relative tolerance of organic pollution. Karr and Chu (1999) suggest expanding the scope of the biological monitoring methods by incorporating several metrics that together provide a greater picture of the stream. The metrics may include identifying taxa richness, functional feeding group, population attributes, and tolerance levels (Karr and Chu 1999). The use of functional feeding groups introduces the use of function as a measure of impact. Our intention is to develop a new metric that can provide additional information on the stream condition based on the biological functioning capacity. This tool could then provide another dimension to the biological assessment, and more importantly, it may provide a more sensitive gauge for understanding nutrient impacts in lotic systems.

Several investigations exist on the effects of elevated nutrients on the stream ecosystem. There have been many studies using different organisms to test contaminant adverse effects of contaminants. Prior to evaluating the adverse impacts, it is necessary to understand the role and function of organisms in the stream. Allan (1995) provides a detailed description of the different trophic levels within the lotic system. He illustrates the complexity of the utilization of organic material. Central to all of the organisms is the link to organic matter and the need to obtain nutrients for energy, growth, and development. We can draw on the results of other studies to provide a basis of understanding of what components affect the nutrient processing in the stream. For example, Richey et al. (1985) showed the effect of primary producers on the nitrogen cycling in the stream. This cycling of nitrogen downstream was influenced by the uptake on the nutrients. In another study, McDougal et al. (1997) illustrated that nutrient enrichment can cause community structure shifts. Further investigation may help to clearly illustrate whether the uptake and processing of material is also altered. Kjeldsen (1996) illustrated the strong link between the primary producers, algae, and their regulation due to invertebrate grazing, phosphorus concentrations, and irradiance. Phosphorus was not found to be a significant controlling mechanism in the primary producers productivity. These field studies provide a basis from which we can build upon in establishing a greater understanding of primary producers' response to phosphorus. There are community level toxicity methods using primary producers that can aid in our effort to understand nutrient enrichment consequences (Blanck 1985). Our studies will be at considerably higher levels of phosphorus than was previously tested, which can then be used to evaluate ambient conditions currently found on the Eastern Shore of Maryland.

Other studies illustrate the processing roles of such organisms as macroinvertebrates and bacteria the stream function (Allan 1995, Fisher et al. 1973, Grimm et al. 1985, Cummins et al. 1973, Wallace et al. 1982, and Petersen et al. 1974). Mulholland et al. (1983, 1985) illustrated that the community structure could alter the phosphorus spiralling length within a stream. Other authors suggest that the processing function of the stream is dependent not only on the organisms utilizing the nutrients but also on the physical characteristics (retention) of the stream (Newbold et al. 1982). They concluded that additional information is needed to understand nutrient transport and the effects on stream function and structure. Although they designed models to predict the effects of organisms individually on the spiraling distance on nutrients, the effects of the entire community as a whole were not considered. We intend to further previous research by investigating what organisms are present in the stream systems, and how they are contributing to the organic processing cycle under high phosphorus conditions. With this information we hope to build an effective assessment tool that can identify the functional capacity of the system to decompose leaf matter under phosphorus rich conditions.

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